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Phyllactinia sebastianiae sp. nov. on *Sebastiania brasiliensis*

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Abstract The new powdery mildew species *Phyllactinia sebastianiae* on *Sebastiania brasiliensis* (*Euphorbiaceae*) is described. This species was collected in Corrientes, Argentina, and is well characterized by conidiophores with twisted foot-cells and dimorphic conidia (lanceolate primary conidia and oblanceolate to ellipsoid-ovoid secondary conidia), indicating an anamorph belonging in the genus *Streptopodium*. Detailed descriptions and illustrations of both anamorph and teleomorph states of the new species are given, and it is compared with morphologically similar taxa.

Key words — *Erysiphales*, taxonomy

Introduction

Recent collections of infected leaves of *Sebastiania brasiliensis* (*Euphorbiaceae*) from local forests of Corrientes, a northeastern province of Argentina, showed severe symptoms of powdery mildew. *Sebastiania brasiliensis* is a native tree of South American subtropical rain forests phytogeographically characterized as "Provincia Paranaense". The region has a warm and humid climate, with precipitations of about 1500 mm per year, more abundant in summer. The medium temperature of the year in this region is 20–21°C (Cabrera 1976). *S. brasiliensis* ("lecheron" or "palo de leche") grows wild in Corrientes in virgin forests, along path edges and ditches, or untilled landscapes. It is a species without timber value but is used as an ornamental due to its conspicuous waxy leaves.

The anamorphic genus *Oidium* Link. seems to be the predominant representative of *Erysiphales* on plants of the *Euphorbiaceae* in the "Paranaense" region. Many species (e.g., *Acalypha wilkesiana* Müll.-Arg., *Croton bonplandianus* Baill., *C. glandulosus* L., *Euphorbia heterophylla* L., *E. hirta* L. var. *hirta*, *E. tithymaloides* L., *Jatropha curcas* L., *J. breviloba* (Morong) Pax &

K. Hoffm., *Manihot esculenta* Crantz, *Microstachys hispida* (Mart.) Govaerts) are infected by *Oidium* spp. These unpublished data supplement the data given in Amano (1986), who listed only *Oidium* spp. and *Phyllactinia suffulta* (Rebent.) Sacc. on *Sebastiania* sp.

Other South American records of *Phyllactinia* Lév. refer to collections on *Erythrina* spp. (*Fabaceae*) and *Morus alba* L. (*Moraceae*) (Mazzanti & Cabrera 1985). Additional South American species (Braun 1987, Havrylenko 1995, Havrylenko et al. 2006) include *Phyllactinia adesmiae* Havryl. on *Adesmia* (*Fabaceae*), *P. ampulliformis* Havryl. on *Discaria* (*Rhamnaceae*), *P. antarctica* Speg. on *Ribes* (*Grossulariaceae*), and *P. chubutiana* Havryl. et al. on *Lycium* (*Solanaceae*) from Argentina and *P. caricaefolia* Viégas on *Carica* (*Caricaceae*) and *P. chorisiae* Viégas on *Chorisia* (*Bombacaceae*) from Brazil.

In this work, a new powdery mildew on *S. brasiliensis* is treated in detail and compared with allied species.

Materials & methods

During 2007 and 2008, leaves showing symptoms of powdery mildew were collected from adult plants in the field. All sites are situated in the province of Corrientes, Argentina (see MATERIAL EXAMINED).

Anatomical observations

The fungal fruiting bodies were carefully scraped from the leaf surfaces with moistened pointed dissection needles and mounted in distilled water. The specimens were then stained and embedded with lactophenol cotton blue (LB) (Gams et al. 1980). Penicillate cells were stained with lactofuchsin (LF) (Carmichael 1955) over a small flame (Shin & Lee 2002). Hyphae, conidiophores, and conidia were stripped from the leaf surfaces with clear adhesive tape and mounted in water, LB, or LF on a microscope slide with the fungal material uppermost. Dried herbarium material was first restored by boiling a small piece of infected leaf in a drop of lactic acid (Shin & La 1993) before being examined as described above. In order to observe the endophytic mycelium, the technique of leaf clearing of Liberato et al. (2005) was used. In each case, fifty measurements of structures of taxonomical value (× 400 magnification) were made, with the average given in parentheses.

CONIDIAL GERMINATION — Conidial germ tubes were observed by incubation on glass slides in damp chambers at room temperature and pressing a sporulating leaf surface directly onto a slide or dislodging conidia by tapping an infected leaf (Boesewinkel 1980, Cook & Braun 2009). In addition, the inoculation on the epidermis of onion scales was used (To-anum et al. 2005). In both cases, the conidia were incubated at 20°–25°C for 24 h until microscopic observation.

LIGHT MICROSCOPY — Micrographs were taken with a NIKON SC 102 light microscope and a camera Sony Cyber-shot 4.1 mpegmovie VX.

SCANNING ELECTRON MICROSCOPY — Fresh leaves with the most suitable areas with powdery mildew symptoms were selected for detailed study. Small pieces about 5 mm^2 were cut out and fixed for 48 h in FAA. The samples were washed, dehydrated, critical

point dried, and sputter coated with gold according to D'Ambrogio de Argüeso (1976). SEM preparations were observed in a JEOL 5800 LV microscope.

Taxonomy

Phyllactinia sebastianiae M.G. Cabrera & Vobis, **sp. nov.** FIGURES 1–6

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Phyllactiniae robiniae *morphologice valde similis, sed conidiophoris ad 287.5 µm longis, conidiis ad 120 × 28 µm, conidiis secundariis oblanceolatis vel ellipsoideis-ovoideis, truncis cellularum penicillatarum chasmotheciorum minoribus, 25 30 × 15 25 µm, simplicibus vel bifurcatis, ascis 2-sporis.*

TYPE: Argentina, Corrientes province, Santa Ana de los Guácaras, on living leaves of *Sebastiania brasiliensis* Spreng. (*Euphorbiaceae*), 6 Jun. 2007, M.G. Cabrera. (Collection N° 442; CTES 0591005, **holotype**; BCRU 05146, isotype).

ETYMOLOGY: derived from the name of the host genus, *Sebastiania*.

SYMPTOMS The symptoms of typical powdery mildew were restricted to the leaves of *Sebastiania brasiliensis* trees. Forming at first rounded to irregular patches of 0.5 to 1 cm in diameter, the fungal parasite developed typical mycelia on both sides of the leaves, predominantly on the adaxial surface (FIG. 1AB). The infections varied and were more abundant on mature than on young leaves. During mycelial development, the leaves changed in color from green to yellow and copper-colored. At a later stage, the most affected areas turned golden-brown (FIG. 1B). The leaves lost their flexibility, dried, and became fragile but kept their flat form. Extensively colonized leaves showed slight chlorosis and later developed a tan necrosis (FIG. 1AB).

The initial mycelia were white, causing a yellowing of the host tissue. Subsequently, the mycelia became denser and changed to yellowish or ash-colored by producing abundant conidia. At this stage, the fungus showed the typical powdery aspect. A hyperparasitic *Cladosporium* sp. frequently slowed down the development of the mycelium, finally destroying it, and darkening the surface of the leaves.

Throughout the year, the foliage of the trees of *S. brasiliensis* was attacked by the powdery mildew in an irregular manner. During the summer, from November to April, the mycelia were scarce, rather opaque, and shriveled. Only slight production of turgescent conidia could be observed. The more remarkable infections were detected during the cold and frosty winter months from May to August, but sometimes also through all seasons, especially on trees growing in shady habitats or in perennially humid environments. In severe attacks, the diseased plants appeared highly deteriorated with completely defoliated branches.

MYCELIUM Two types of mycelia developed: external and internal.

The ectophytic mycelium is amphigenous, at first delicate, later becoming dense, effuse at maturity, moderately thick and subpersistent, more compressed

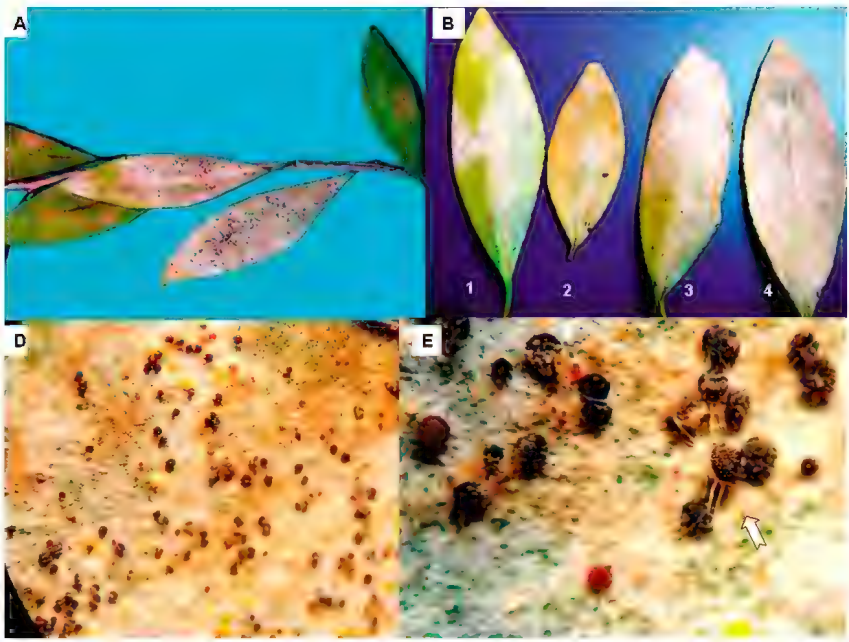

FIGURE 1. *Phyllactinia sebastianiae* aspect. A. Powdery mildew symptoms and occurrence of chasmothecia on *S. brasiliensis* leaves. B. Mycelia covering the adaxial surfaces (1 and 3), and abaxial surfaces (2 and 4). C. Group of immature and mature chasmothecia on mycelium. D. Chasmothecium with acicular appendages

with age. The mycelium is initially whitish, later yellow. Finally, the external mycelium covers the whole surface of the leaf (FIG. 1AB). The ectophytic hyphae are almost straight to sinuous, sometimes geniculate, hyaline, thin-walled, smooth and septate, branched, with long cells, 44(72.5)140 × 3.75(5.8)8 µm (FIG. 3CD). Appressoria are solitary, variable in shape, ranging from nipple-shaped, lobed to almost coralloid to hooked or slightly forked (FIGS. 3F 6E). The appressoria are generally not very abundant.

In addition, a typical endophytic mycelium is developed (FIG. 3C). In transverse sections of the leaf, hyaline, branched hyphae were observable within the host plant tissue (FIG. 5A). Globose haustoria formed terminally on short hyphal side branches (FIG. 5B). Ectophytic and endophytic mycelia are connected by hyphae emerging through the stomata (FIG. 3C).

CONIDIOPHORES The anamorphic structures are characterized by hyaline conidiophores, which arise directly from the external mycelium (FIG. 3D). They are 75(158)287 µm long and 5(6.78)8 µm wide. The basal septa are situated above the junctions with the supporting ectophytic hyphae, up to about 7.5 µm

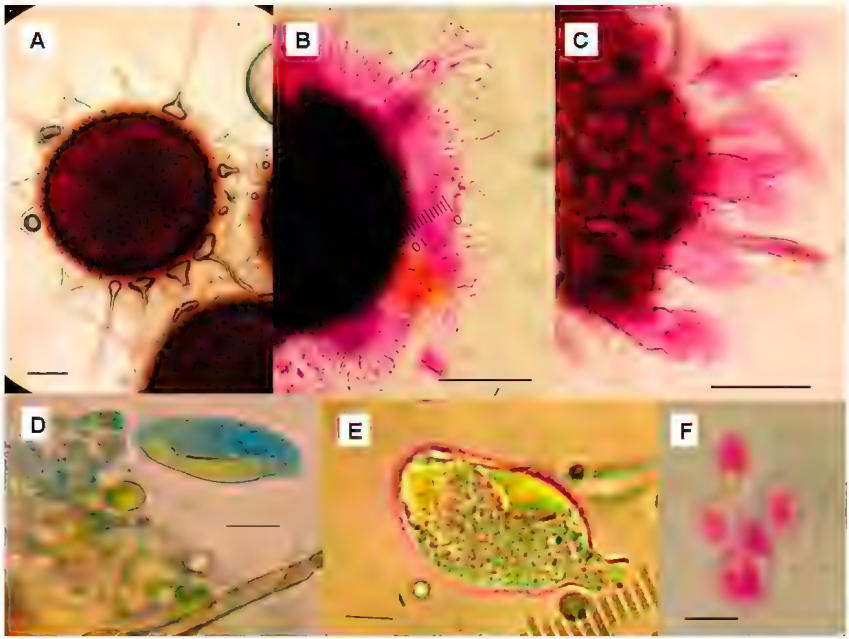

FIGURE 2. Characteristics of *Phyllacinia sebastianiae* conidia. AC. Primary conidia. DE. Secondary conidia. Germ tube development in B, C and E. (Bar: 25 µm)

(FIG. 6E). The long foot-cell, 37(158)250 × 5(6.78)8 µm, is twisted in 1–3 loops or sinuous at its base (FIG. 3D) and followed by 1 or 2 shorter cells (FIG. 6E).

CONIDIA The conidia are developed singly at the apex of the conidiophore (FIG. 6E). Sometimes two conidia are produced successively. They are one-celled and hyaline with thin walls. Young conidia are slightly rough, becoming rougher or slightly reticulate when mature (FIGS. 2DE 3E). In young conidia, the cytoplasm is hyaline with abundant vacuoles of different sizes (FIG. 2A). Oil drops can also be observed (FIG. 2B). Two types of conidia are produced (FIG. 6E). The primary conidia are lanceolate and apically pointed (FIGS. 2AC 4E), whereas the secondary conidia are more oblanceolate to ellipsoid-ovoid (FIGS. 2DE 3F). Primary and secondary conidia have similar sizes (57.5(78)120 × 14(24)28 µm).

CONIDIAL GERMINATION The germ tubes are usually produced in a subapical position, sometimes also laterally or at both apices. They are 70–95 µm long and develop unlobed or multilobed appressoria (FIGS. 2BCE 3F 6E). Under natural conditions, the germination rate for both primary and secondary conidia was moderate (70%). Laboratory assays demonstrated a lower germination rate, 23% for the incubation method on glass slides, and 29% for inoculation on onion scales.

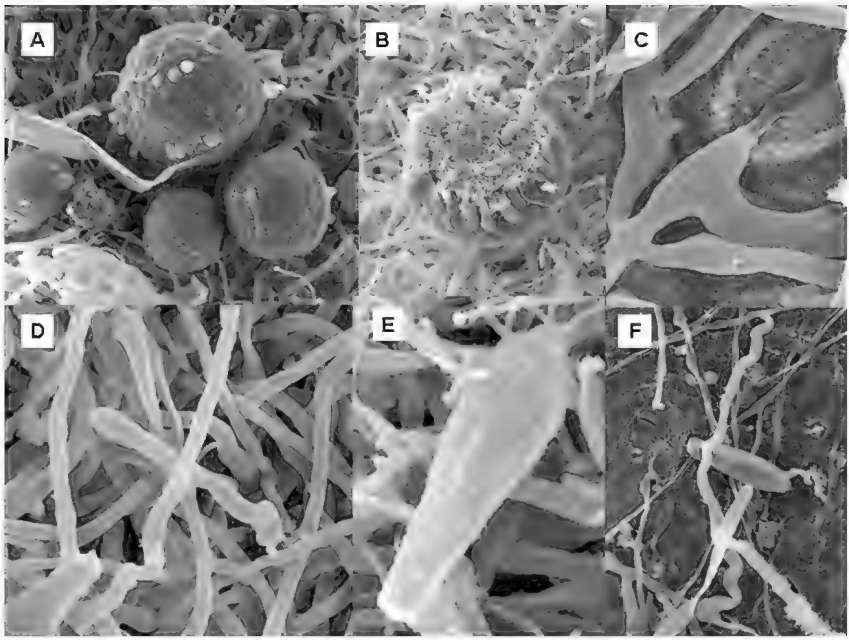

FIGURE 3. Teleomorphic structures of *Phyllactinia sebastianiae* (holotype). A. Anatomical structures of ascomata. B. Superficial vision of numerous filaments and penicillate cell. C. Peridial cells and basal penicillate cell D- Immature ascus and ascospores E. Pedicellate immature ascus. F. Immature ascospores. ((Bar: A,B, 50 µm; C,F. 25 µm; D. 15 µm; E. 12 µm)

CHASMOTHECIA The chasmothecial ascomata are gregarious or scattered on both surfaces of the leaves (FIG. 1AC). They are spherical when young (FIG. 4A), becoming later distinctly depressed at the lower side (FIG. 1D). The color of the initials of the fruiting bodies is white, changing to pale yellow and orange, and finally turns dark brown or nearly black when mature (FIG. 1CD). The well developed chasmothecia have diameter of 142(180)250 µm. The peridium consists of polygonal cells with thick and dark walls. (FIGS. 3A 4A 6A). They measure 6.25(10)12.5 µm in diameter.

APPENDAGES Each individual fruiting body presents two types of appendages: acicular appendages and penicillate cells.

The acicular appendages are arranged in equatorial position. Each chasmothecium bears 9(17)22 acicular appendages (FIG. 3A), which are rigid, hyaline, with thick walls, having a crystalline or vitreous appearance (FIG. 1D). Their bases are swollen, forming a 27.5(32.5)40 µm diam., globular structure, and the apexes are pointed (FIG. 6AC). The acicular appendages are variably long (107(195)312.5 µm), generally exceeding the diameter of the chasmothecium.

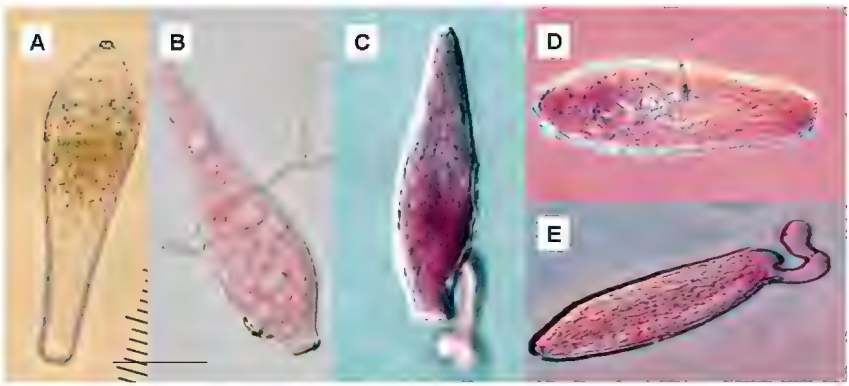

Figure 4. SEM Micrographs of *Phyllactinia sebastianiae*. A. Development of penicillate cells on chasmothecia. B. Young penicillate cells and acicular appendages of immature ascoma (500×). C. Hypha protruding from a stoma (2000×). D. Conidiophores and hyphae of external mycelium and conidiophores (1000×). E. Rough conidium outer surface (1700×). F. Germ tube with appressorium of secondary conidium (700×)

The penicillate cells, which are located on the upper side of the ascoma (Figs. 3B 4B), are composed of a stem that bears free thin filaments (Figs. 4B 6B) and is connected to the peridium and protrudes from the surface of the ascoma wall (Fig. 4C). The stem is roughly cylindrical, 25–30 × 15–25 µm, occasionally bifurcate in its upper part and extending into numerous, thin, 25–70 µm long filaments. The stem walls are thin and the filaments are embedded in a mucilaginous mass that covers completely the top of the chasmothecium (Fig. 3B).

Asci and ascospores Various specimens, collected from the same sites during different periods over two years, were investigated, but material with totally mature asci was never observed. The description is based on collections obtained in winter, the most favourable season for growth and development of reproductive structures. The asci are arranged in clusters inside the ascoma. They are broadly clavate to globoid, with well-developed pedicels (Figs. 4DE 6D).

The ascus walls are thin and hyaline. The asci are 50–57.5 × 32.5–37.5 µm. It is estimated that about 10% of the asci produce ascospores under the above characterized natural conditions. A number of 2-3 ascospores per ascus perhaps can be observed. The ascospores are hyaline, ellipsoidal to ovoid (Fig. 6D), having a dense cytoplasm, which contains 2-3 yellow oil drops (Figs. 2F). They are 25–37.5 µm long and 18–22.5 µm wide.

Additional specimen examined: ARGENTINA. Corrientes Province, Santa Ana de los Guácaras, near National Route 12 (S 27°2'; W 58°23'), 4 June 2007, R.E. Álvarez N° 441 (CTES 0591004).

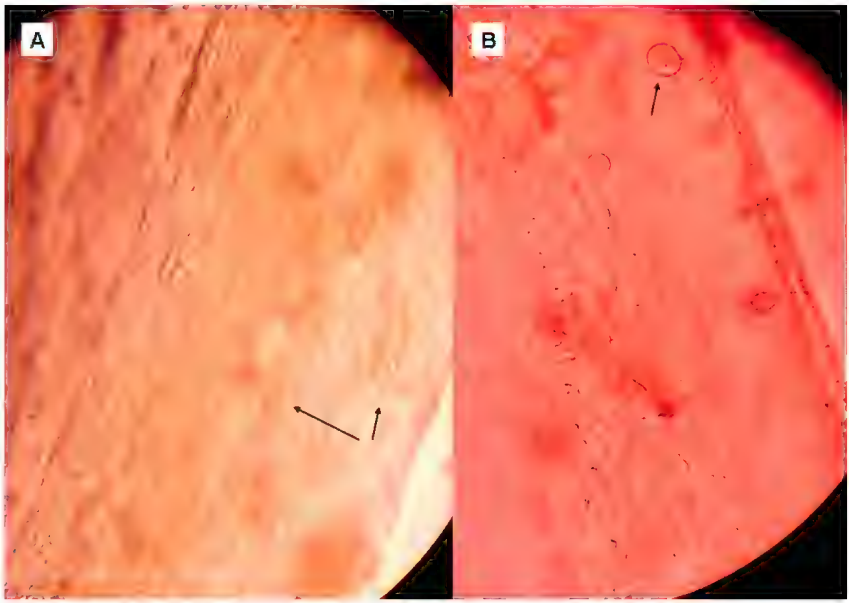

FIGURE 5. *Phyllactinia sebastianiae* A. Endophytic hyphae within *S. brasiliensis* leaf. B. Formation of haustoria.

Discussion

Although the median temperature of the year in the Paranaense phytogeographical region is 20–21°C (Cabrera 1976), in the province of Corrientes the temperature ranges from –2.8°C to 42.4°C (median temperature 26.8°C) (Sánchez et al. 2007). This thermic variation is important for its influence on chasmothecial maturity. During the growing season the fungus produces mainly the anamorphic stage. Ascospores were usually immature.

The fungus exhibits the typical anamorphic morphology of *Streptopodium* R.Y. Zheng & G.Q. Chen as circumscribed by Braun (1987), characterized by a hemiendophytic mycelium that enters the leaf tissue through stomata (FIG. 3C) and dimorphic conidia (FIG. 6E). The conidia outer walls are verrucose when viewed by SEM (FIG. 3E), and the conidiophores have sinuous bases (FIG. 3DF). Ascoma characters agree with those of the genus *Phyllactinia*.

Amano (1986) registered *Oidium* sp. and *Phyllactinia suffulta* on *Sebastiania* sp. However, the anamorph of *P. guttata* (Wallr.) Lév. (the current name for *P. suffulta*) belongs to *Ovulariopsis* Pat. & Har. (Takamatsu et al. 2008), which is characterized by straight conidiophores. The anamorph of the fungus we observed on *S. brasiliensis* does not belong to *Ovulariopsis*, and the teleomorph is not conspecific with *P. guttata* (Braun 1987). Furthermore, the conidiophore foot-cells are spirally twisted or sinuous. Several *Phyllactinia* spp. with twisted

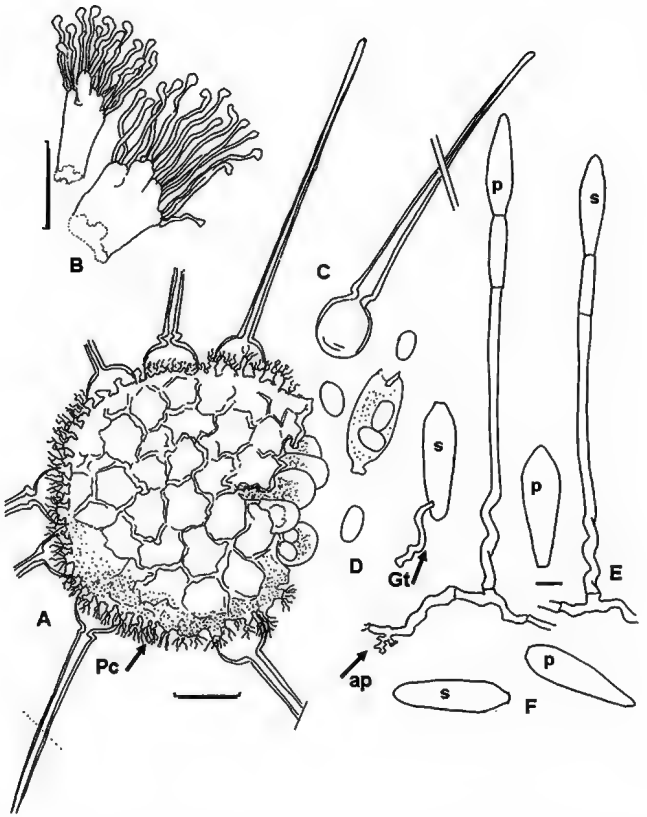

FIGURE 6. *Phyllactinia sebastianiae* (holotype). A. Chasmothecium with peridial cells and appendages. B. Numerous filaments on penicillate cells. C. Acicular appendage with swollen base. D. Pedicellate ascus and ascospores. E. Conidiophores with sinuous basal cells. F. Primary and secondary conidia. (Bar: A, 20 µm; B, 40 µm; C F, 20 µm)

conidiophore foot-cells and dimorphic conidia are known, but all of them inhabit hosts of unrelated families. *Phyllactinia robiniae* U. Braun & Yáñez-Morales is morphologically close to *P. sebastianiae* but differs in having shorter (70–130 µm) conidiophores, narrower (40–70 × 12–22 µm) clavate secondary conidia, penicillate cells with narrower stems (25–50 × 8–18 µm), several short terminal branchlets, and 2-spored asci (Braun & Yáñez-Morales 2009). *Phyllactinia erythrinae-americanae* Yáñez-Morales & U. Braun (Braun & Yáñez-Morales 2009) is distinguished by its conidiophores with basal septa elevated 10–35 µm above the junction with the supporting hypha. Furthermore, the twisted part of the conidiophores is usually confined to the portion below the basal septum. The *Phyllactinia* sp. on *Erythrina* spp. in Argentina (Mazzanti de Castañón &

Cabrera de Álvarez 1985) is morphologically rather close to the latter species but differs in having larger chasmothecia with a larger number of appendages. The taxonomy of these collections from Argentina is not yet clear (Braun & Yáñez-Morales 2009). *Phyllactinia caricaefolia* on *Carica papaya* in Brazil is also characterized by forming dimorphic conidia but this species is easily distinguishable from *P. sebastianiae* by its much smaller ascomata and distinctly clavate secondary conidia (Viégas 1944, Liberato et al. 2004). *Streptopodium caricae* Liberato & R.W. Barreto (Liberato et al. 2004) is probably the anamorph of *P. caricaefolia*. *Phyllactinia chorisiae* on *Chorisia speciosa* in Brazil is another species with dimorphic conidia, but the conidiophores are straight and the secondary conidia distinctly clavate (Liberato 2007). *Phyllactinia dalbergiae* Piroz. on hosts of the *Fabaceae* possesses conidiophores with twisted foot-cells, but the conidia are uniformly clavate (Mukerji 1968, Braun 1987).

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Literature cited

Amano (Hirata) K 1986. Host range and geographical distribution of the powdery mildews fungi. Japan Scientific Societies Press, Tokyo. 741 p.

Boesewinkel HJ. 1980. The morphology of imperfect states of powdery mildews (*Erysiphaceae*). Botanical Review 46(2):167–224. doi. 10.1007/BF02860869

Braun U 1987. A monograph of the *Erysiphales* (powdery mildews). Beihefte zur Nova Hedwigia 89: 1–700.

Braun U Yáñez-Morales M 2009. *Phyllactinia* and *Ovulariopsis* species on legumes. Mycotaxon 109: 145–160.

Cabrera AL. 1976. Regiones fitogeográficas Argentinas. Enciclopedia Argentina de Agricultura y Jardinería 2(1): 1–85. Kluger WF (ed). ACME Buenos Aires.

Carmichael JW. 1955. Lacto-fuchsin: A new medium for mounting fungi. Mycologia 47: 611.

Cook RTA, Braun U. 2009. Conidial germination patterns in powdery mildews. Mycological Research 113: 616–636. doi: 10.1016/j.mycres.2009.01.010

D`Ambrogio de Argüeso A. 1986. Técnicas de preparación del material para ser observado con MEB (microscopio electrónico de barrido). 60–63, in: Manual de Técnicas en Histología Vegetal. Editorial Hemisferio Sur SA. Buenos Aires, Argentina.

Gams W, Aa HA van der, Plaats-Niterink AJ van der, Samson RA & Stalpers JA. CBS Course of Mycology. 2nd. ed. 1980. Centraalbureau voor Schimmelcultures Baarn (The Netherlands). ERLA, Amsterdam-Zuid.

Havrylenko M, Takamatsu S, Divarangkoon R, Braun U. 2006. *Phyllactinia chubutiana*: a new species of Erysiphales from Patagonia (Argentina). Mycoscience 47: 237–241. doi:10.1007s10267-006-0301-0

Havrylenko M, Takamatsu S. 2005. Notes on *Erysiphales* (*Ascomycetes*) from Patagonia, Argentina. Mycoscience 46: 32-38. doi: 10.1007/s 10267-004-0209-5

Liberato JR. 2007. Taxonomic notes on two powdery mildews: *Phyllactinia chorisiae* and *Ovulariopsis wissadulae* (*Erysiphaceae*: *Phyllactinieae*). Mycotaxon 101: 29–34.

Liberato JR, Barreto RW, Louro RP. 2004. *Streptopodium caricae* sp. nov., with a discussion of powdery mildews on papaya, and emended descriptions of the genus *Streptopodium* and *Oidium caricae*. Mycological Research 108: 1185–1194. doi: 10.1017/S0953756204000991

Liberato JR, Barreto RW, RG Shivas. 2005. Short Research Notes. Leaf-clearing and staining techniques for the observation of conidiophores in the *Phyllactinoideae (Erysiphaceae)*. Australasian Plant Pathology 34: 401–404.

Mazzanti de Castañón MA, Cabrera de Álvarez MG. 1985. Estudio de oídios (formas sexuales y asexuales) registrados sobre cinco especies ornamentales en Corrientes, Argentina. 2° Congreso Latinoamericano de Fitopatología. Tomo I. Micología book of abstracts. Buenos Aires: 303–312.

Mukerji KG. 1968. *Phyllactinia dalbergiae*. C.M.I. descriptions of pathogenic fungi and bacteria. N° 186. Eastern Press Ltd., London.

Sánchez JE, Aleman JA, Prosdocimi A, (eds.). 2008. Atlas total clarín de la Republica Argentina. Corrientes. Arte Grafico Editorial Argentino SA.Buenos Aires. Tomo 11.

Shin HD, La YJ. 1993. Morphology of edge lines of chained immature conidia on conidiophores in powdery mildews fungi and their taxonomic significance. Mycotaxon 46: 445–451.

Shin HD, Lee HJ. 2002. Morphology of penicillate cells in the genus *Phyllactinia* and its taxonomic application. Mycotaxon vol. 83: 301–325.

Takamatsu S, Inagaki M, Niinomi S, Khodaparast SA, Shin HD, Grigaliunaite B, Havrylenko M. 2008. Comprehensive molecular phylogenetic analysis and evolution of the genus *Phyllactinia* (*Ascomycota: Erysiphales*) and its allied genera. Mycological Research 112: 299–315 doi: 10.1016/jmycres.2007.11014

To anun C, Kom un S, Sunawan A, Sato Y, Takamatsu S. 2005. A new subgenus, *Microidium*, of *Oidium* (Erysiphaceae) on *Phyllanthus*. Mycoscience 46: 1–8. doi: 10.1007/s10267-004-0202-z

Viégas AP. 1944. Alguns fungos do Brasil II. Ascomycetes. Bragantia 4(1–6): 1–392.